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The first aim identifies prostatic stem cells b a novel in vivo approach using our normal prostatic basal and luminal cell lines. The second aim studies the characteristics of prostatic tumors arising after transformation of these cells. We established a novel intraprostatic transplantation assay in which GFP-tagged basal cells engraft and differentiate into luminal cells. The basal cells also differentiate into luminal cells when inoculated under the renal capsule (RC). This indicates that these cells have stem cell features as they self-renew and give rise to mature luminal progeny in two in vivo assays. We also show that transformed basal and luminal cells form subcutaneous tumors and that the rate of tumor growth is enhanced by normal prostatic smooth muscle (SM) cells. This indicates that SM may contribute to the progression of prostatic tumors and that paracreine signaling between epithelial and SM cells may promote carcinogenesis. Intraprostatic or sub-RC inoculation of transformed basal cells gives rise to tumors that contain luminal cells. This indicates for the first time that tumorigenic basal cells (arising form transformed stem cells) can give rise to prostatic tumors with a luminal phenotype. This is relevant as it explains how prostate cancer could originate in basal stem cells yet manifest clinically as a tumor expressing luminal features.

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Introduction

This is a final report that covers the period 10/01/2000-09/30/2003 and will therefore be a summary of the purpose and results of the research completed during this time.

The aim of this proposal was to identify prostatic stem cells by a novel in vivo approach using prostatic basal and luminal cell lines that we have established in culture (1, 2). The stem cells of any organ renew the tissue throughout the lifespan of the individual. They are long-lived cells that divide infrequently. Most of the cell division in an organ takes place in the post-stem cell compartment, the transit-amplifying (TA) cell population (3, 4). The identification and characterization of stem cells is important since they represent a major target of carcinogenesis as well as a potential source of benign prostatic hyperplasia (BPH) (5, 6). The location of prostatic stem cells is currently unknown. They are generally considered to lie within the basal compartment (7, 8) although some evidence indicates that luminal cells may also have stem cell properties (9, 10). An important feature of stem cells is their ability to engraft in their appropriate niche. *The first aim* of this proposal was to transfect our normal murine cell lines with GFP and to determine whether the basal and luminal cells can engraft intraprostatically in a novel prostate transplant assay that we proposed developing. Evidence of engraftment in vivo would indicate that the cell lines had features of stem cells. We were able to show that GFP-tagged basal cells give rise to luminal cells in two in vivo assays. *The second aim* was to study the characteristics of prostatic tumors that arise after transformation of basal and luminal cells. The cell lines were transformed with the *ras* oncogene and the composition and characteristics of the tumors were examined. We wished to determine whether transformed basal cells could give rise to luminal cells as most prostatic tumors have a luminal phenotype. As the stem cell compartment is considered to reside in the basal layer (7, 8, 11) and as tumors may originate from the transformation of stem cells (6) it is puzzling that most prostatic tumors express a luminal phenotype. Using *ras* transformed basal cells we have shown that these cells gave rise to tumors that contained both basal and luminal cells indicating that transformed basal cells can give rise to transformed luminal cells in vivo.

Body

Aim 1. This aim examined the ability of our prostatic cell lines to behave as stem cells and to engraft and form prostatic tissues in vivo.

Tasks 1-3. The normal basal and luminal prostate cell lines were transfected with GFP and their behavior studied in vitro and in vivo. Some of these results have been described in two publications that are attached to this proposal (12, 13).

A characteristic of stem cells is their ability to home, engraft and proliferate in their 'niche' within their compartment. Definitive identification of stem cells requires functional in vivo assays that permit engraftment of tagged candidate stem cells that produce differentiated progeny. We created a stem cell niche in the prostate and have shown that GFP-tagged donor cells engraft, proliferate and differentiate into luminal cells within this niche. The niche was created by castrating 4-week old C57Bl/6 mice and allowing prostate involution for 20 days. Our cloned basal epithelial cell line was tagged by stably transfecting the cells with the vector pTracer-SV40 (Invitrogen, CA), that allows GFP expression by the cells. The GFP-basal line

was inoculated into 'primed' involuted prostates of animals that had received a subcutaneous androgen pellet 24 hr prior to cell inoculation. Once the prostate had regenerated (14 days), animals were cycled by removing and adding androgen to amplify any GFP-expressing cells that had incorporated into ducts. Paraformaldehyde fixed tissue was examined immunohistochemically using a specific antibody to GFP (Invitrogen). **Fig. 1** shows that the cloned GFP-labeled basal cells incorporated into some prostatic ducts and gave rise to GFP-labeled luminal cells. This indicates that prostatic basal cells can incorporate into prostatic ducts and give rise to luminal cells in the first example of an in vivo prostate transplant model.

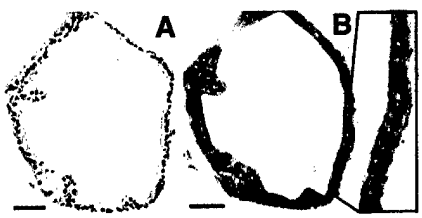

Fig. 1. GFP-expressing basal cells engraft in the regenerating prostate. GFP-basal cells (2.5 x 10^5 cells/lobe/10 μl) were inoculated into involuted prostates of castrated mice given androgens 24 hr prior to inoculation to stimulate growth. **(A)** Section using a control antibody. **(B)** Engrafted cells were detected using an anti-GFP-antibody and a secondary HRP-conjugated antibody with Vector Nova Red as substrate. GFP-basal cells were incorporated into the ducts and gave rise to GFP-luminal cells. Insert-high magnification of section of duct. Bars=70 μm.

To quantitate the overall level of engraftment we examined digests of prostates of animals using immunocytochemical methods for GFP and basal cytokeratin expression after the animals had been cycled for 1, 2 or 3 cycles by adding and removing androgens. This was done to determine if androgen cycling increased the numbers of engrafted cells. We found that the numbers of GFP-expressing basal cells increased from approximately 1% after 1 cycle to 5% after 2 cycles and to 28% after 3 cycles indicating progressive amplification of the engrafted GFP-basal cell line under these conditions.

The basal and luminal cell lines were also inoculated under the renal capsule (RC) in the absence and presence of our prostate smooth muscle (SM) cell line (1) in intact and castrated animals to study stromal/epithelial interactions in vivo. These results have been published and the manuscript is attached (13). They indicate that prostatic tissue containing stromal and epithelial elements including ductal elements (See Fig. 2, (13)) was generated under the RC when the basal and luminal cell lines were inoculated with SM cells whereas none of the lines formed significant tissue when inoculated alone (See Fig. 1, (13)). The luminal cell line produced prostatic secretory products specific to that of the dorsolateral prostate from which the line was isolated (See Fig. 2G,H, (13)). Castration reduced the amount of tissue formed when basal and SM cells were co-inoculated, indicating that the growth of this tissue is androgen responsive (See Fig. 3, (13)).

The basal cell line was also inoculated under the RC in the presence of urogenital sinus mesenchyme (UGM) which is known to promote prostate growth and development. Sections of this tissue indicated that the basal cell line could form prostatic ducts that contained both basal and luminal cells indicating that basal cells could differentiate into luminal cells under the RC **(Fig. 2).**

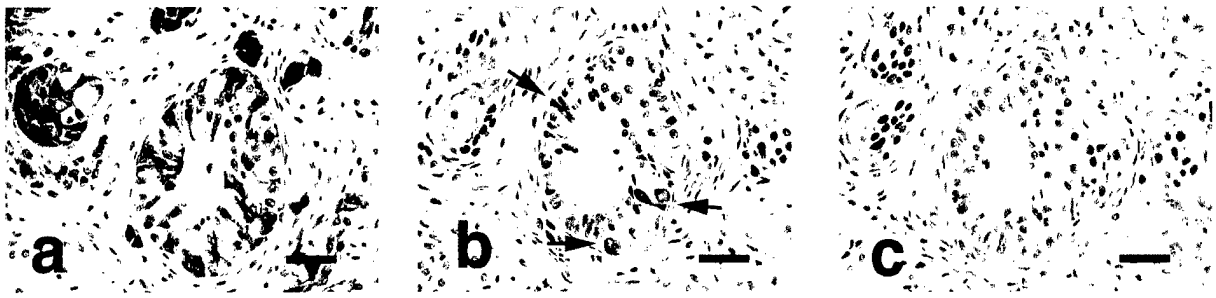

Fig. 2. GFP-expressing basal cells form prostatic ducts containing basal and luminal cells under the renal capsule (RC). GFP-expressing basal cells (10^5 cells) were inoculated with urogenital sinus mesenchyme cells ($2.5x10^5$ cells) under the RC. After 12 weeks the resulting tissue was examined immunohistochemically for cytokeratin expression. (a) Basal cells gave rise to prostatic ductal tissue expressing basal cytokeratins (CK 5). (b) The ductal tissue arising from basal cells also contained cells of luminal origin (CK 8; arrows). (c) Control sections using normal IgG gave no staining confirming the specificity of the CK 5 and CK 8 staining. Bars = 100μm.

These results indicate that our normal basal cell line has stem cell features as it engrafts into a regenerating prostate, proliferates and gives rise to mature luminal cells both intraprostatically and under the renal capsule.

Aim 2. This aim examined the characteristics of tumors that arose from transformed basal and luminal cell lines and determined if transformed basal cells could give rise to tumors containing luminal cells.

Tasks 4-8. Our prostatic basal and luminal cell lines were transformed and GFP-tagged using an N-rasV12 construct and their properties examined both in vitro and in vivo. Some of these results have been published in an attached manuscript (12).

We studied four transformed cell lines (one basal and three luminal) and all formed subcutaneous tumors (See Fig.4, (12)). None of the lines was metastatic. The lines formed tumors that grew equally in intact and castrated mice indicating that they were not androgen sensitive. The rate of subcutaneous tumor formation of the transformed basal cell line (TE-B-1) and one of the luminal cell lines (TE-L-4) was considerably enhanced by the simultaneous inoculation of prostatic smooth muscle cells (see Fig. 5, (12)). As prostatic smooth muscle tightly envelops the epithelium this may indicate that smooth muscle may interact with adjacent epithelium to promote prostatic tumor growth. One of the transformed luminal cell lines (TE-L-4) gave rise to tumors that contained ductal structures strongly expressing luminal cytokeratin 8. (See Fig. 6D, E, (12)). The basal cell line also formed tumors when inoculated intraprostatically and under the renal capsule. These cells were more tumorigenic when inoculated intraprostatically than subcutaneously with tumor volumes approximately 2.7 ± 0.2 g for intraprostatic tumors vs 0.3 ± 0.1 g for subcutaneous tumors and 1.2 ± 0.2 g for sub-renal capsule tumors **(Fig. 3).**

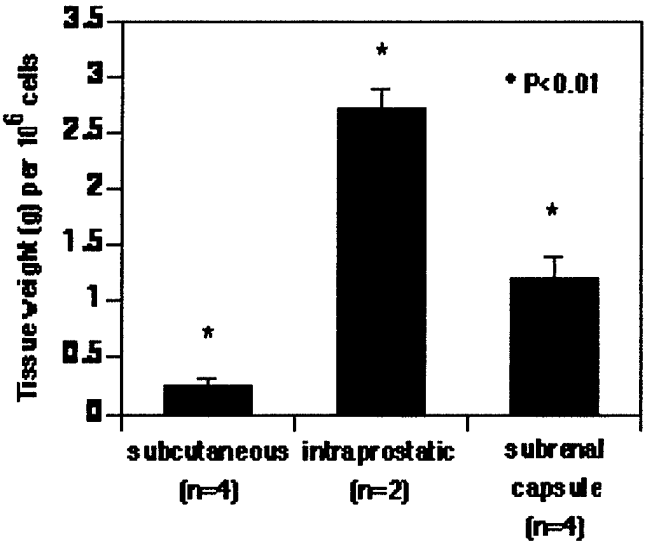

Fig. 3. *Ras*-transformed basal cells formed tumors in vivo. *Ras*-transformed basal cells were inoculated subcutaneously, intraprostatically and under the renal capsule and animals were sacrificed after 15 days. Tumor tissue was removed and weighed.

A puzzling feature of prostate carcinoma is the fact that most human prostate tumors have a luminal rather than a basal phenotype. If the stem cells reside in the basal compartment (7, 8, 11) and our data (see results in Aim 1) and if tumors arise from the transformation of stem cells (6) then prostate carcinoma would be expected to have a basal phenotype. We therefore determined if transformed basal cells could give rise to cells with luminal features. Both intraprostatic (See Fig. 7, (12)) and sub renal capsule (**Fig. 4**) tumors contained cells expressing luminal features (as evidenced by the presence of luminal cytokeratin 8). This indicates that tumors with a luminal phenotype may arise from transformed basal 'stem' cells. It is therefore possible that by the time prostate cancer is diagnosed the luminal cells may have proliferated to such an extent that the basal origin of the tumor is obscured. This may explain how a transformation event taking place in a basal 'stem' cell can result in a prostate tumor whose cells express predominantly luminal rather than basal cytokeratins.

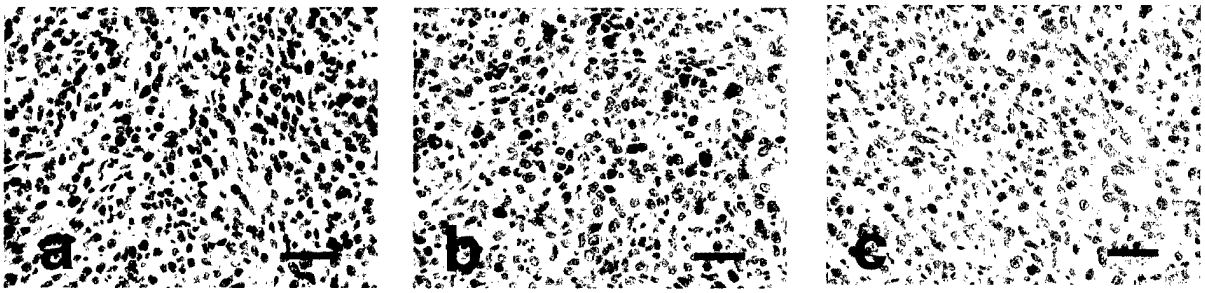

Fig. 4. GFP-expressing *ras*-transformed basal cells form tumors containing basal and luminal cells under the renal capsule (RC). GFP-expressing *ras*-transformed basal cells ($3x10^6$ cells) were inoculated under the RC. The tumor tissue was examined immunohistochemically for cytokeratin expression. (a) *Ras*-transformed basal cells gave rise to tumors expressing basal cytokeratins (CK 5). (b) The tumor tissue arising from transformed basal cells also contained cells of luminal origin (CK 8). (c) Control sections using normal IgG gave no staining confirming the specificity of the CK 5 and CK 8 staining. Bars = 100μm.

Key research accomplishments

1) Generation of normal GFP-tagged prostatic basal and luminal cell lines.
2) Generation of a panel of *ras*-transformed GFP-expressing basal (one cell line) and luminal cell lines (two *ras*-transformed, one spontaneously transformed) that are tumorigenic in vivo.
3) A novel intraprostatic transplantation model in which our normal basal cell line engrafts, multiplies and differentiates has been established.
4) Normal basal cells have been shown to differentiate into luminal cells in two in vivo assay – the intraprostatic transplantation assay and also in a sub-renal capsule assay. This indicates that this basal cell line is useful as a stem cell model as it regenerates itself and gives rise to differentiated progeny.
5) Normal prostatic smooth muscle cells promote the growth of some of the tumor cell lines in vivo. This indicates that prostatic smooth muscle cells adjacent to tumor cells may interact with them and enhance their tumorigenic potential.
6) Intraprostatic and sub-renal capsule inoculation of *ras*-transformed basal cells give tumors that contain both basal and luminal cells. This indicates for the first time that tumorigenic basal cells (arising from transformed stem cells) can give rise to prostatic tumors than contain cells with luminal features.

Reportable outcomes

1) We have developed a panel of GFP-expressing normal and *ras*-transformed basal and luminal prostatic cell lines that are available for distribution.
2) Some of the results of this work have been published in two manuscripts (12, 13). See appendix.
3) Sarah Salm applied successfully for a DOD postdoctoral fellowship based partially on some early data obtained from this project.

Conclusions

These results are important for prostate carcinogenesis for a number of reasons. Prostate tumor cells and normal stem cells have many common features. These include infinite life span, androgen independence, multi-drug resistance and telomerase expression. It is therefore evident that the isolation and culture of prostate stem cells may enable the design of rational new therapies to treat prostate carcinoma. Our results show that a normal prostate basal cell line can engraft intraprostatically and under the renal capsule and give rise to luminal cells indicating that this basal cell line has stem cell features. This cell line will therefore be a valuable resource in exploring the manner whereby basal stem cells differentiate into luminal secretory cells and the mechanisms by which this process is regulated.

In addition, we have created a panel of transformed prostate cell lines that are tumorigenic in vivo and that have a number of special features of interest. A transformed luminal cell line (TE-L-4) that forms tumors with a differentiated phenotype containing ducts lined by cells expressing luminal cytokeratins will be useful for determining mechanisms whereby some tumors maintain differentiated functions whereas others express only undifferentiated features.

The most interesting finding to emerge from our studies is that transformed basal cells can give rise to tumors that contain luminal cells. As prostate stem cells reside in the basal layer and as basal stem cells are the likely targets of transformation it has been puzzling that most prostate tumors have a luminal phenotype. Our results may explain this paradox as transformed basal cells may give rise to tumorigenic luminal cells. By the time prostate cancer is clinically evident most of the basal cells within the tumor may have been replaced by cells with luminal features.

We have also shown that prostate smooth muscle cells can promote the growth of prostate tumors. This indicates that smooth muscle cells may contribute to the progression of prostate tumors and that paracrine signaling between basal and smooth muscle cells may be involved in this process.

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E Lynette Wilson. PI. 35% effort
Sarah Salm. Post doctoral fellow. 100% effort. On grant until 01/15/02 when she received a DOD post-doctoral award.
Sandra Coetzee. Research Associate. 100% effort. On grant as from 01/15/02.

Manuscripts supported by this grant – see appendix

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T. Takao, A. Tsujimura, S. Coetzee, S. Salm, H. Lepor, E. Shapiro, D. Moscatelli, E. L. Wilson. Stromal/epithelial interactions of murine prostatic cell lines in vivo: a model for benign prostatic hyperplasia and the effect of doxazosin on tissue size. The Prostate 54: 17-24 (2003).

Appendix

The Prostate 54:17–24 (2003)

Stromal/Epithelial Interactions of Murine Prostatic Cell Lines In Vivo: A Model for Benign Prostatic Hyperplasia and the Effect of Doxazosin on Tissue Size

Tetsuya Takao,[1,2,3] Akira Tsujimura,[1,2,3] Sandra Coetzee,[1] Sarah N. Salm,[1] Herbert Lepor,[3,4] Ellen Shapiro,[3,4] David Moscatelli,[1,4] and E. Lynette Wilson[1,3,4]*

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BACKGROUND. One of the major constraints in elucidating the mechanisms involved in the etiology of benign prostatic hyperplasia (BPH) is the lack of suitable model systems that are readily manipulable in vitro and in vivo. To address this issue, we have used murine prostatic cell lines to establish a novel in vivo model for studying prostatic cell interactions.
METHODS. Luminal, basal, and smooth muscle (SM) cell lines were inoculated alone or in combinations under the renal capsule of intact or castrated male mice, and the growth and composition of prostatic tissue in the absence or presence of doxazosin was determined.
RESULTS. Both the luminal and basal cell lines reconstituted prostatic tissue if co-inoculated under the renal capsule with normal SM cells, whereas none of the lines formed significant tissue when inoculated alone. Luminal cells produced and secreted prostatic secretory products. The growth of prostatic tissue formed from co-inoculation of basal and SM cells was androgen responsive. In addition, a significant reduction in prostatic tissue was noted in animals treated with doxazosin.
CONCLUSION. We have established an in vivo model that uses prostatic epithelial and SM cell lines for investigating cellular interactions between epithelial and SM cells that regulate prostatic growth and function. This model will be useful for delineating the mechanisms by which prostatic cells interact and in determining the efficacy of new approaches aimed at interfering with prostatic stromal/epithelial interactions that result in abnormal cellular proliferation. *Prostate 54: 17–24, 2003.* © 2002 Wiley-Liss, Inc.

KEY WORDS: **benign prostatic hyperplasia; prostatic cell lines; androgens; doxazosin**

INTRODUCTION

Although benign prostatic hyperplasia (BPH) occurs with high frequency in the aging human male, its exact etiology is currently unknown [1,2]. It is a disease in which dysregulation of the prostatic stroma results in an increase in the stromal to epithelial ratio from 2:1 to 5:1 and in which both epithelial and stromal compartments of the prostate proliferate [3,4]. In the adult, prostatic smooth muscle (SM) produces a variety of growth factors that influence the behavior of the adjacent epithelium [5–8]. BPH may develop from an imbalance

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between cell proliferation and cell death, which may result from perturbations in cellular interactions between the prostatic stroma and its adjacent epithelium [9,10] or from a reawakening of the inductive potential of the adult mesenchyme [11,12]. It has also been proposed that aberrant stem cell proliferation may contribute to the development of BPH [13].

One of the major constraints in understanding the underlying causes of BPH is the lack of suitable model systems to investigate the etiology of this disease. There are only a limited number of animal models for studying BPH. Canines can be used for these purposes, as they develop naturally occurring and experimentally induced androgen-sensitive prostatic hyperplasia [14,15]. Recently, the spontaneously hypertensive rat was shown to develop adenomatous BPH with a mild increase in prostatic stromal elements [16]. In addition, male transgenic animals in which int-2 (a member of the fibroblast growth factor family) is overexpressed develop an epithelial BPH that is hormonally sensitive [17]. A mouse prostate reconstitution model in which transforming growth factor beta (TGF-β) is overexpressed results in hyperplastic lesions that resemble BPH [18,19]. In addition, the combination of urogenital sinus mesenchyme with epithelial cells provides a good experimental system for delineating the epithelial-mesenchymal interactions that may be involved in abnormal proliferation of prostatic compartments [5–8]. We considered that one of the deficiencies hampering the ability to delineate mechanisms involved in prostatic stromal/epithelial interactions was the lack of a panel of normal hormonally sensitive prostatic cell lines from an inbred strain of mice that could be used both in vitro and in vivo to study cellular interactions. To address this deficiency, we have established several prostatic cell lines from p53-deficient animals that include a smooth muscle (PSMC1), a basal (PE-B-1), and a luminal (PE-L-1) cell line [20–22]. We find that little growth of prostatic tissue results when each individual cell line is engrafted under the renal capsule, whereas both the luminal and basal cell lines reconstitute prostatic tissue in the presence of normal SM cells. As doxazosin, an α1-adrenergic antagonist, has been shown to inhibit growth by inducing apoptosis in an experimental BPH system [23] and in prostate cancer [24], we determined its effects on tissue growth under the renal capsule. Doxazosin was observed to diminish the amount of prostatic tissue formed in vivo.

MATERIALS AND METHODS

Cell Lines and Engraftment Under the Renal Capsule

Prostatic smooth muscle (PSMC1), luminal (PE-L-1), and basal (PE-B-1) cell lines were established from p53 null C57BL/6 mice and cultured as previously described [20–22]. In some instances, cell lines (PSMC1, PE-L-1, PE-B-1) that had been stably transfected with the expression tracer vector pTracer-SV40 (Invitrogen, Carlsbad, CA), which contains a green fluorescent protein (GFP)-Zeocin fusion gene, were used to verify that prostatic tissue was of donor origin.

Cells were cultured in their appropriate media and harvested by trypsin treatment from culture dishes when approximately 80% confluent. Cell combinations were prepared by mixing 2.5×10^5 basal or luminal cells with 5×10^5 SM cells. When basal, luminal, and SM cells were combined, 1.25×10^5 basal, 1.25×10^5 luminal, and 5×10^5 SM cells were mixed. Each cell type was also grafted alone by using 7.5×10^5 cells. Cells were pelleted and resuspended in 30 μl of type1 collagen (Vitrogen-100 collagen from Collagen Corporation, Palo Alto, CA). The collagen was allowed to gel at 37°C for 15 min, and the gels were grafted beneath the renal capsule of 6-week-old male C57BL/6 mice (Taconic, Germantown, NY) [25] (tutorial for technique: http://mammary.nih.gov/tools/Cunha001/index.html).

To determine the effect of androgen on tissue growth, collagen gels were implanted into intact animals and those that had been castrated 9 days before gel implantation. In these instances, 1×10^6 basal or luminal cells together with 5×10^5 SM cells were implanted in collagen gels. To determine the effect of doxazosin on tissue growth, collagen gels containing 1×10^6 basal, 1×10^6 luminal, and 6.7×10^5 SM cells were implanted under the renal capsule. Each cell combination was implanted under the renal capsule into at least five kidneys for each experiment. Doxazosin or sterile water was administered intraperitoneally at 3 μg/g body weight/day for 6 weeks. Previous data indicate that this dosage was effective in a mouse prostate reconstitution model [23]. In addition, complete blockade of α1-adrenoceptors of rodent cells are achieved at this dosage as the effective dose in rodents is approximately 0.3 μg/g. Toxic effects are not anticipated from the dose we used as the lethal dose is in excess of 200 μg/g [26,27]. Animals were killed between 4 and 6 weeks after gel implantation and the dimensions of each graft were measured. Tissue volume was calculated by the formula (length × width × width × 1/2) [28].

Immunohistochemistry

Kidneys were fixed in 70% ethanol or 4% paraformaldehyde and immunohistochemistry was performed as described previously [20–22]. Mouse monoclonal antibody to cytokeratin 8 (Progen Immuno-Diagnostica, Heidelberg, Germany) and cytokeratin 14 (YLEM,

Rome, Italy) were directly coupled to horseradish peroxidase (HRP) by using Dako's Envision + kit (Dako, Carpinteria, CA) and detected by using DAB as the substrate. Rabbit polyclonal antibodies to secretory products that are specific to the dorsal prostate were a gift from Dr. C. Abate-Shen (Piscataway, NJ; Kim, Shen, and Abate-Shen, manuscript in preparation). A wide spectrum anticytokeratin rabbit polyclonal immunoglobulin (Ig) G was a gift from Dr. T.-T. Sun (New York, NY). Antibodies to α-smooth muscle actin (Sigma Chemical Company, St. Louis, MO) and GFP (Invitrogen) were detected by using appropriate secondary HRP-linked antibodies (Amersham Life Science, Inc., Arlington, IL) or appropriate secondary biotinylated antibodies (Dako; Vector, Burlingame, CA) by using the Vectastain Elite ABC kit (Vector). Nonimmune serum or appropriate IgG was used in place of primary antibodies for controls. Sections of prostatic tissue or tissue obtained from a transgenic mouse expressing GFP in all tissues (Jackson Laboratories, Bar Harbor, ME) were used as positive controls for all antibodies.

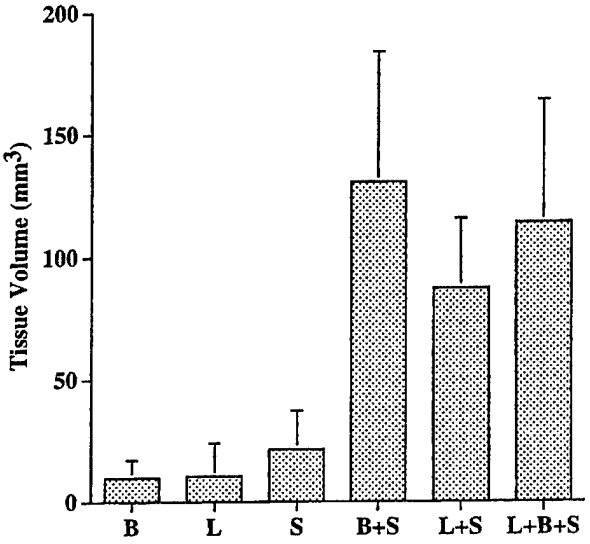

Fig. 1. Epithelial/smooth muscle interactions promote the formation of prostatic tissue under the renal capsule. Collagen gels containing basal cells (B); luminal cells (L); smooth muscle cells (S); basal and smooth muscle cells (B + S); luminal and smooth muscle cells (L + S); and luminal, basal, and smooth muscle cells (L + B + S) were inoculated under the renal capsule. Animals were killed 5 weeks later, and tissue volume was measured. Each bar represents the mean ± SD of the tissues from six kidneys (five kidneys in the case of smooth muscle cells).

RESULTS

Co-Inoculation of SM Cells With Basal or Luminal Epithelial Cells Promotes Tissue Growth In Vivo

As stromal/epithelial interactions are important for the development and function of the prostate [29–31], we determined the effects of co-inoculation of the luminal or basal epithelial cells with the SM cell line under the renal capsule. A small amount of prostatic tissue was found when the basal, luminal, or SM cells were inoculated alone (9.8 ± 7.5, 10.4 ± 13.9, and 21.6 ± 15.8 mm^3, respectively) (Fig. 1). In contrast, striking growth of prostatic tissue was noted when either basal or luminal epithelial cells (2.5×10^5 cells/mouse) were inoculated together with prostatic SM cells (5.0×10^5 cells/mouse). More than five times as much prostatic tissue was obtained when the epithelial cells were co-inoculated with SM cells compared with the amount of tissue formed when each cell type was inoculated alone ($P < 0.01$). The amount of tissue obtained when all three lines were inoculated together (114.0 ± 50.1 mm^3) was not significantly different from that found when either epithelial cell line was co-inoculated with the SM cell line (B + S = 130.4 ± 53.5 mm^3; L + S = 87.5 ± 28.3 mm^3). Histologic examination of the prostatic tissue masses that were obtained after co-inoculation of luminal and basal epithelial cells with SM cells revealed in some instances structures that resembled prostatic ducts (Fig. 2A,B) that were lined by cells expressing luminal (CK 8) (Fig. 2C,D,F) and basal (CK 14) (Fig. 2E,F) cytokeratins and that were surrounded by stromal tissue containing SM cells (Fig. 2M,N). In some instances the lumen of ducts contained secretory material. This material and the luminal epithelial cells contained secretory products specific to that of the dorsal prostate (Fig. 1G,H) (the lines were established from the dorsolateral prostate of p53 null mice). The cells producing the secretory products expressed luminal CK 8 (Fig. 2I,J). This finding indicates that, in this prostatic model system, luminal epithelial/stromal interactions occur that result in the production of functional secretory products by the luminal epithelial cells of the ducts. Staining of regions of duct-free stromal tissue with pancytokeratin antibodies indicated that they also contained epithelial cells (Fig. 2K,L), although these cells did not express either luminal or basal cytokeratins (data not shown). As the cell lines expressed either basal (PE-B-1) or luminal (P-LE-1) cytokeratins in vitro [20–22], this finding indicates that they may have lost their specific cytokeratin expression but not their epithelial character when located in regions of tissue that did not contain ductal elements. Basal and luminal cytokeratins may only be expressed when epithelial cells are in a ductal orientation. Random tissue sections through transplants showed that approximately

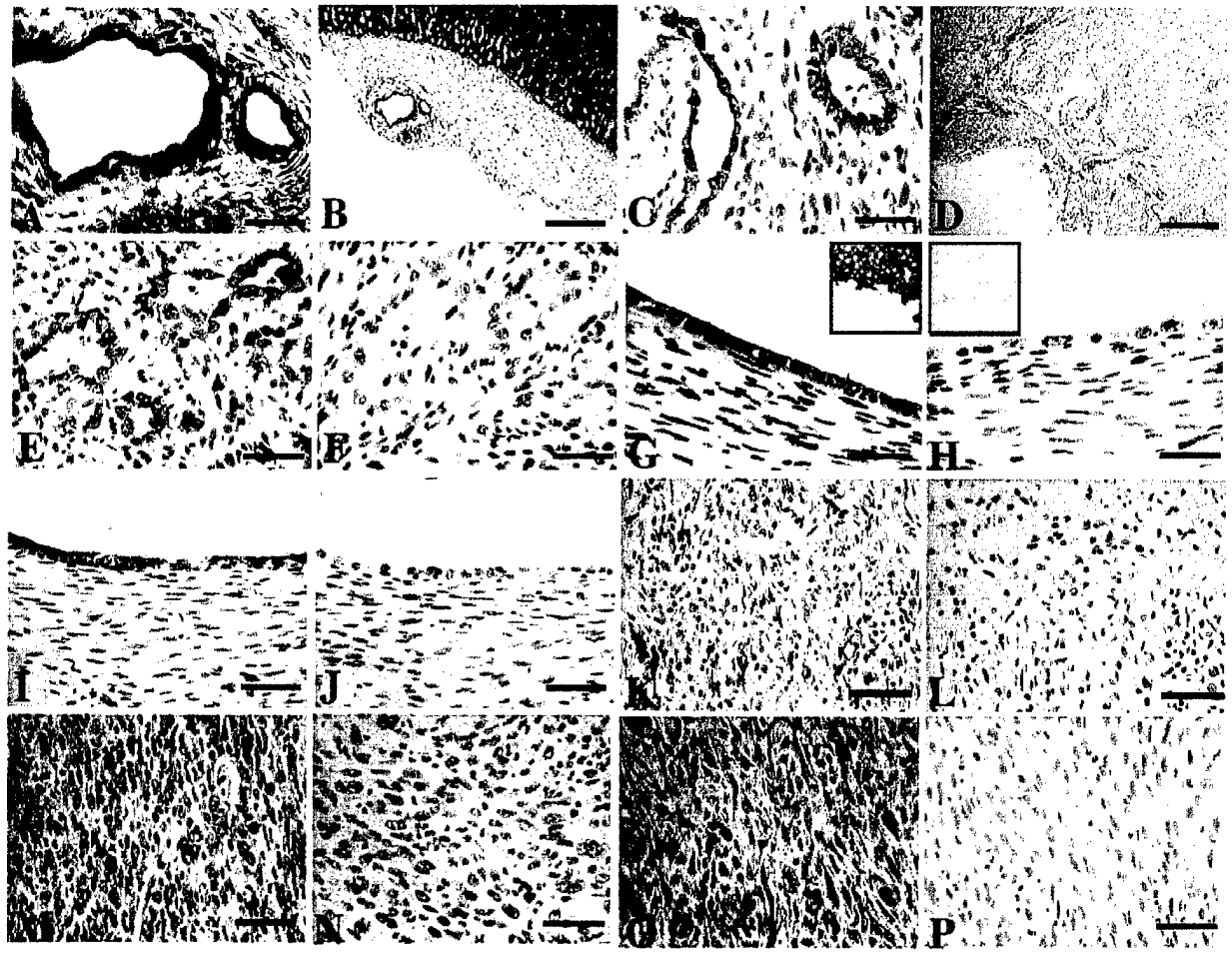

Fig. 2. Histologic properties of tissue derived from the co-inoculation of epithelial and smooth muscle cells. **A:** A paraffin section of prostatic tissue stained with hematoxylin and eosin arising from the co-inoculation of basal (PE-B-1), luminal (PE-L-1), and smooth muscle (SM; PSMC1) cell lines. **B:** A paraffin section of prostatic tissue stained with hematoxylin and eosin showing a low-power view of the field depicted in A. **C:** A paraffin section of prostatic tissue arising from inoculation of all three lines showing luminal cells surrounding ducts stained by using a horseradish peroxidase (HRP)-conjugated murine antibody against K8 keratin and diaminobenzidine (DAB) as substrate. **D:** A paraffin section of prostatic tissue showing a low-power view of the field depicted in C. **E:** A paraffin section of prostatic tissue arising from inoculation of all three lines showing basal cells surrounding ducts stained by using an HRP-conjugated murine antibody against K14 keratin and DAB as substrate. **F:** A paraffin section of prostatic tissue arising from inoculation of all three lines stained with HRP-conjugated normal mouse immunoglobulin (Ig) G indicated that the cytokeratin staining in C, D, and E was specific. **G:** A paraffin section of prostatic tissue arising from inoculation of luminal and SM cells showing that the ductal epithelial cells contain and secrete (boxed inset) prostatic products specific to the mouse dorsal prostate. Sections were stained with rabbit antibodies specific for dorsal prostatic secretory products and visualized with secondary goat anti-rabbit HRP-conjugated antibodies with DAB as substrate. **H:** A paraffin section and secretory material (boxed inset) stained with normal rabbit serum indicated that the staining in G was specific. **I:** A paraffin section of prostatic tissue arising from inoculation of luminal and SM cells showing CK 8-expressing luminal cells. **J:** A paraffin section of prostatic tissue arising from inoculation of luminal and SM cells stained with HRP-conjugated normal mouse IgG indicated that the CK 8 staining in I was specific. **K:** A paraffin section of prostatic tissue arising from inoculation of all three lines showing cytokeratin expressing cells stained with a wide spectrum rabbit anti-cytokeratin IgG and visualized with a secondary goat anti-rabbit biotinylated antibody by using the Vectastain Elite ABC kit. **L:** A paraffin section of prostatic tissue arising from inoculation of all three lines stained with rabbit IgG and visualized with a secondary goat anti-rabbit biotinylated antibody by using the Vectastain Elite ABC kit, indicated that the staining in K was specific. **M:** A paraffin section of prostatic tissue arising from inoculation of all three lines showing SM expressing cells stained with a murine anti-α smooth muscle actin antibody and visualized with a secondary goat anti-mouse HRP-conjugated antibody with NovaRed as substrate. **N:** A paraffin section of prostatic tissue arising from inoculation of all three lines stained with a normal murine antibody and visualized with a secondary goat anti-mouse HRP-conjugated antibody with NovaRed as substrate indicated that the staining in M was specific. **O:** A paraffin section of prostatic tissue arising from inoculation of green fluorescent protein (GFP) -transfected basal, luminal, and SM cell lines showing GFP-expressing cells stained with a rabbit anti-GFP antibody visualized with a secondary goat anti-rabbit biotinylated antibody by using NovaRed as substrate. **P:** A paraffin section of prostatic tissue arising from inoculation of GFP-transfected basal, luminal, and SM cell lines stained by using normal rabbit serum visualized with a secondary goat anti-rabbit biotinylated antibody by using NovaRed as substrate indicated that the staining in O was specific. Scale bars = 50 μm in A,C,E–P, 300 μm in B,D.

10–15% of the transplants showed evidence of gland formation. The majority of the tissue is composed of stromal cells and consists of a mixture of stromal and epithelial cells (Fig. 2K) in a ratio of 3:1, with relatively few glandular cells present (Fig. 2B,D). Thus, in this system, stromal growth requires the presence of an epithelial element. When GFP-transfected cell lines were inoculated, the prostatic tissue expressed GFP (Fig. 2O,P), indicating that the tissue was of donor origin.

As all three cell lines expressed androgen receptor protein and as androgens stimulated the growth of the basal and SM cell lines in vitro [20–22], we determined the effects of androgens on the growth of prostatic tissue in vivo. The growth of tissue derived from mixtures of basal and SM cells was significantly less in castrated animals than in intact animals (Fig. 3; $P < 0.05$), whereas although less growth of tissue was observed in castrated animals that received mixtures of luminal and SM cells, the differences were not significant (Fig. 3; $P = 0.19$). These results indicate that the growth of tissue derived from mixtures of basal and SM cell lines was responsive to androgens in vivo.

These results show that both the luminal and basal epithelial cell lines reconstitute prostatic tissue when inoculated under the renal capsule in the presence of normal prostatic SM cells, whereas none of the lines form significant prostatic tissue when inoculated alone. In addition, luminal cells are functionally active as they produce prostatic secretory products. Moreover, the growth of tissue obtained from basal and SM cell combinations is androgen responsive.

Doxazosin Inhibits Growth of Prostatic Tissue In Vivo

As doxazosin, an α1-adrenergic receptor antagonist, is administered to alleviate the symptoms of BPH, we determined its effect on the amount of prostatic tissue derived from combinations of basal, luminal, and SM cells. We found that significantly less prostatic tissue was obtained in animals treated with doxazosin compared with that noted in control animals (Fig. 4; $P < 0.05$). The histologic composition of the tissue was similar in both groups of animals.

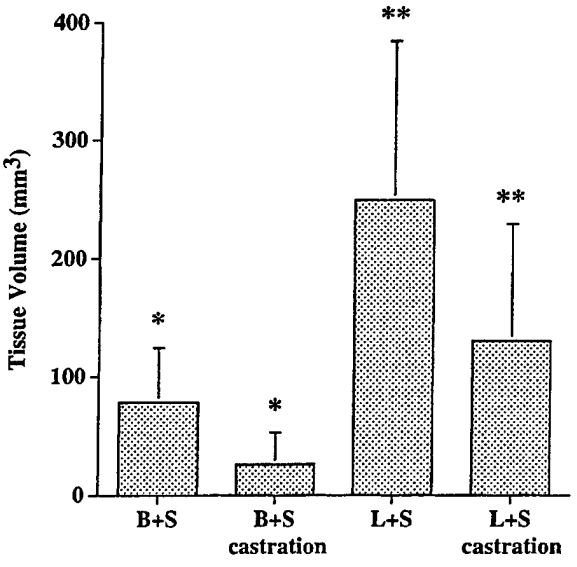

Fig. 3. The effect of androgens on the growth of prostatic tissue under the renal capsule. Basal and smooth muscle (B + S) and luminal and smooth muscle (L + S) cells were inoculated under the renal capsule of intact and castrated animals. Animals were killed 4 weeks later, and tissue volume was measured. Significantly less tissue was present in castrated animals receiving basal and SM cells (*$P < 0.05$). There was no significant difference in prostatic tissue in intact or castrated animals inoculated with luminal and SM cells (**$P = 0.019$). Each bar represents the mean ± SD of the prostate tissues from five kidneys.

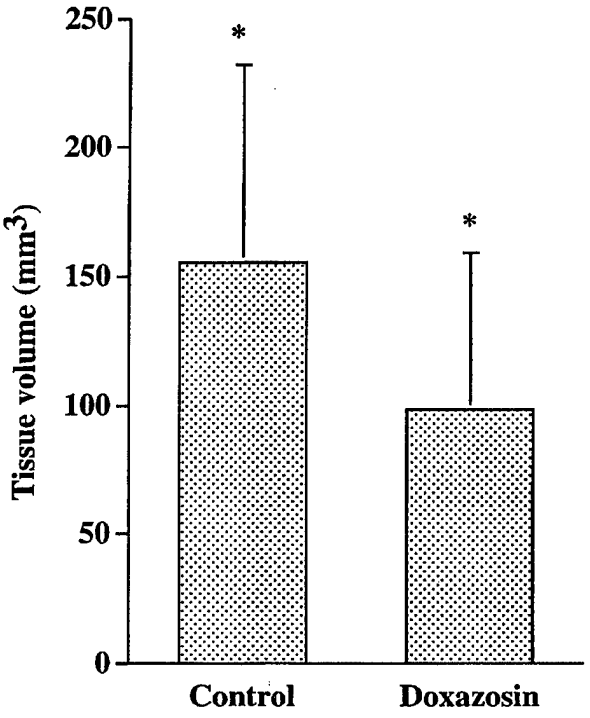

Fig. 4. Doxazosin reduces the amount of prostatic tissue under the renal capsule. Basal, luminal, and smooth muscle cells were inoculated under the renal capsule of control animals and those receiving doxazosin. Significantly less prostatic tissue was present in doxazosin-treated animals (*$P < 0.05$). Each bar represents the mean ± SD of the tissues from 18 kidneys.

DISCUSSION

We have shown that both basal and luminal epithelial cell lines formed prostatic tissue under the renal capsule when combined with a prostatic SM cell line, whereas these lines formed very little tissue when inoculated alone. Luminal cells also produced prostatic secretory material. In addition, the amount of tissue formed when basal epithelial and SM cells were combined was androgen sensitive, as less tissue was noted in castrated animals. The administration of the α1-adrenergic antagonist, doxazosin, resulted in significantly less tissue under the renal capsule. To the best of our knowledge, these cell lines represent the first panel of prostatic cell lines that are capable of reconstituting prostatic tissue in vivo in a syngeneic mouse model system. The rat basal cell line NRP-152 has been shown to differentiate into prostatic ductal structures containing luminal cells secreting prostatic products when inoculated with urogenital mesenchyme (UGM) under the renal capsule in immunodeficient mice, whereas no glandular architecture was noted when cells were inoculated with 10T1/2 fibroblasts [25]. Studies using UGM in place of the prostatic SM cell line are planned to determine whether the inductive influence of UGM differs from that of the SM cell line.

An interesting finding is that the amount of tissue formed from combinations of basal and SM cell lines was diminished in castrated animals, indicating that tissue formation was androgen sensitive. Although all three cell lines express androgen receptor protein, only the basal and SM cell lines are androgen responsive in vitro [20,21]. The SM cells produce significant amounts of TGF-β, which stimulates their growth in an autocrine manner, and dihydrotestosterone increases proliferation of the SM cells by promoting TGF-β secretion [21]. It is possible, therefore, that a dynamic interaction between the androgen receptor axis and the TGF-β signaling pathway may regulate growth in this in vivo model system. Similar interactions may be involved in the abnormal growth that accompanies prostatic hyperplasia. Co-culture of luminal and SM cells in vitro increased the amounts of active TGF-β generated by the cell lines [20], indicating that one of the components mediating the increased stromal growth noted when combinations of epithelial and SM cells are inoculated under the renal capsule may be increased levels of active TGF-β generated by epithelial/SM interactions. TGF-β accumulates at mesenchymal/epithelial interfaces during mouse prostate development [32] and in a TGF-β-transduced mouse prostate reconstitution model, TGF-β accumulation was observed in areas of benign hyperplasia [18]. There are reports that TGF-β may be either stimulatory [33,34] or inhibitory [35–37] to the growth of prostatic stromal cells. Some of this discrepancy may be related to the amounts of TGF-β used in these studies, as low levels (0.01 ng/ml) of TGF-β have been shown to stimulate growth of human prostatic stromal cells, whereas higher levels (1 ng/ml) have been shown to inhibit growth [34]. As TGF-β is produced in a latent form that requires activation before binding to its signaling receptors [38,39] it is possible that the increased stromal element noted in BPH could result from aberrant stromal/epithelial interactions in BPH that result in generation of levels of active TGF-β, which promote growth of stromal tissue.

Doxazosin significantly inhibited the amount of prostatic tissue formed by epithelial/stromal interactions in our subrenal capsule model of prostatic cellular interactions. Sympathetic regulation of SM tone in the prostate and bladder neck by means of α1-adrenergic receptors is considered to be relevant in the urethral obstruction associated with BPH, and α1-adrenergic antagonists, such as doxazosin, that inhibit SM contractility have been used for the treatment of urinary symptoms associated with BPH. Doxazosin has also been reported to have effects on apoptosis that are independent of α1-adrenergic receptor expression, and it may induce stromal regression in BPH by both α1-adrenergic–dependent and –independent pathways [24,40,41].

CONCLUSIONS

By using epithelial and SM cell lines established from p53 null mice, we have established a novel in vivo model for investigating the interactions between prostatic SM and epithelial cells. We show that prostatic tissue containing epithelial and stromal elements is generated under the renal capsule when epithelial cell lines are inoculated with SM cells, whereas none of the cell lines form significant amounts of tissue when inoculated alone. Luminal cells produce and secrete prostatic secretory products specific to that of the dorsolateral prostate. Castration reduces the amount of tissue formed when basal and SM cells are co-inoculated, indicating that the growth of this tissue is androgen responsive. Finally, the α1-adrenergic antagonist doxazosin diminishes the amount of prostatic tissue generated, indicating that this model may be useful for identifying promising therapeutic agents for treating BPH.

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The Prostate 51:175 – 188 (2002)

Differentiation and Stromal-Induced Growth Promotion of Murine Prostatic Tumors

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BACKGROUND. We have derived a panel of p53-null prostatic "basal" and "luminal" epithelial cell lines and their ras transformed counterparts to study stromal/epithelial interactions and the properties of tumors arising from "basal" and "luminal" cells.
METHODS. Previously derived normal murine prostatic "basal" epithelial (PE-B-1) and "luminal" epithelial (PE-L-1) cell lines were transformed with N-Ras. These lines and a spontaneously transformed "luminal" cell line were inoculated subcutaneously or orthotopically into athymic mice, alone or in combination with normal prostatic smooth muscle cells (SMC).
RESULTS. All transformed lines formed subcutaneous tumors. SMC significantly enhanced the growth rate of the tumors arising from the "basal" and one of the "luminal" cell lines. The transformed "basal" line gave rise to tumors expressing both "basal" and "luminal" cytokeratins.
CONCLUSIONS. Prostatic SMC promote the growth of transformed epithelial cells, suggesting that prostatic stroma may promote tumor development. Furthermore, transformed "basal" cells give rise to tumors containing "luminal" cells, suggesting that although most human tumors have a "luminal" phenotype, they may originate from transformed "basal" cells. *Prostate 51: 175–188, 2002.* © 2002 Wiley-Liss, Inc.

KEY WORDS: **prostate; epithelial; transformed; cell lines; tumors**

INTRODUCTION

Prostate cancer is a major health concern that represents an increasingly large burden on health resources in the Western world [1]. However, despite the magnitude of this problem, progress in prostate disease research has been limited by the lack of adequate animal models that reproduce the spectrum of human prostate disease, namely benign, latent, aggressive, and metastatic forms [2]. Several groups have developed human prostate cancer cell lines [2–8] and xenografts [9,10] which have been used as model systems for the examination of the process of malignant transformation. However, most prostatic cell lines are derived from metastatic prostate cancer and are appropriate for studying advanced progression of the disease and treatment [11]. To our knowledge there are no reports of normal murine prostatic cell lines that have transformed counterparts and that can be used to examine the manner in which such cells differ in their growth responses in vitro and in vivo.

Ras genes comprise a highly conserved ubiquitous eukaryotic gene family that plays a fundamental role

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in normal cellular proliferation [12]. Mammalian *ras* genes can be activated to acquire transformation-inducing properties by single point mutations in their coding sequence [13–15]. Transformation of primary cells generally requires the introduction of two complementary oncogenes such as *ras* and *myc* [16] and may also require inactivation or mutation of tumor suppresser genes such as *p53* [17–20]. However, established immortalized rodent cell lines are readily transformed by the introduction of a single activated *ras* oncogene [21,22]. In addition it has been shown that the introduction of *ras* into animals with a defective *p53* gene results in a dramatic increase in the malignant features of the transformed cells and in matastasis [23].

The murine prostatic epithelium is comprised of basal cells and secretory luminal cells. Prostate stem cells are thought to reside in the basal layer and it is proposed that the basal cells give rise to the luminal cells via an intermediate cell type that expresses both basal and luminal characteristics [24–30]. Recent supporting evidence for this comes from a rat basal cell line that has been shown to give rise to luminal cells both in vitro [31] and in vivo [32]. The nature of the cells that serve as the targets of transformation in the prostate that give rise to prostatic carcinoma are currently unknown. However, prostatic tumor cells usually have a luminal phenotype [33–35], suggesting that if transformation takes place in a basal cell, it must give rise to luminal cells that undergo unlimited proliferation. Alternatively the luminal cells may be transformed directly. As yet, it is unclear which process occurs in prostatic carcinoma.

There is compelling evidence to support the concept that a functional stromal response contributes to cancer progression [36]. Previous reports have indicated that stromal cells stimulate tumor progression [36–40]. Paracrine signaling between other cell types and carcinoma cells plays an important role in tumor formation, affecting efficiency of tumor formation, tumor growth rate and size, invasiveness of the tumor, and development of metastases [41].

We have recently established a panel of murine prostatic cell lines from p53 null mice that represents the major cell types in prostatic tissue [42,43]. To study the behavior of transformed prostatic basal and luminal cells in vivo and in vitro we transformed our panel of normal "basal" and "luminal" cell lines using a *ras* oncogene, thereby creating transformed counterparts to these lines. We found that the growth of two of the cell lines was significantly enhanced by smooth muscle cells (SMC) in vivo and that the transformed "basal" cell line gave rise to cells with "luminal" features when inoculated intraprostatically.

MATERIALS AND METHODS

Materials

Collagenase was obtained from Sigma Chemical Company, St. Louis, MO, (Type II, C-6885), Vitrogen-100 collagen from Collagen Corporation, Paolo Alto, CA, Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium from Gibco BRL, Rockville, MD, and fetal bovine serum (FBS) from Intergen Co, Purchase, NY.

Cell culture media contained the following additives from Sigma Chemical Company: insulin, cholera toxin, hydrocortisone, transferrin, phosphoethanolamine, dihydrotestosterone (DHT), glutamine, and bovine serum albumin (BSA). Other additives included fibroblast growth factor-2 (Scios Nova, Sunnyvale, CA), epidermal growth factor (Genzyme, Cambridge, MA), and bovine pituitary extract (Gibco BRL).

Antibodies to basal cytokeratins 5 and 14 (BL18 and LL001) and luminal cytokeratins 8 and 18 (LE41 and LE61) were a gift from Prof. E.B. Lane (Dundee, Scotland). A wide spectrum anti-cytokeratin polyclonal antibody was a gift from T-T Sun (New York, NY). Monoclonal antibody to cytokeratin 8 was also obtained from Progen Immuno-Diagnostica, Heidelberg, Germany. This antibody was directly coupled with HRP using Dako's Envision + kit (Dako, Carpinteria, CA). FITC-labeled goat anti-rabbit and goat anti-mouse antibodies were from Sigma Chemical Company and Southern Biotechnology Associates, Birmingham, AL, respectively. TGF-β neutralizing antibody, 1D11.16 was from Genzyme. The antibody to green fluorescent protein (GFP) was from Invitrogen, Carlsbad, CA. HRP-linked goat anti-mouse and rabbit were obtained from Amersham Life Science, Inc., Arlington, IL.

Animals

Athymic mice from the National Institutes of Health were used for all in vivo experiments. Mice were housed in a climate-controlled facility and all animal care and procedures were performed in compliance with IACUC guidelines.

Cell Culture

Normal prostatic cell lines were established from p53 null C57BL/6 mice [42,43]. These lines included two smooth muscle cell lines (PSMC1 [42] and PSMC2), a "basal" epithelial cell line (PE-B-1) and a "luminal" epithelial cell line (PE-L-1) [43]. The SMC lines were cultured in DMEM with 10% FBS. The normal "basal" and "luminal" cell lines were transfected with the N-RasV12 proto-oncogene to generate tumorigenic counterparts, as described below. The "basal" and "luminal" epithelial cell lines were grown

in serum-containing and serum-free media respectively, with additives as described previously [43].

Transfection With Green Fluorescent Protein (GFP) and N-RasV12 Construct

Cells were transfected with expression vector pTracer-SV40 (Invitrogen) which has a GFP tag and into which we cloned a N-Ras proto-oncogene, with a site-directed V12 mutation, creating vector pTracer-SV40-N-RasV12. The *ras* oncogene was amplified from vector pS65-N-RasV12 (provided by A. Pellicer, New York, NY) using the following primers: 5′ GAA TCC AGG ATG GCC ATG ACT GAG TAC AAA CTG GTG GTG and 3′ GCG GCC GCT TAC ATC ACC ACA CAT GGC AAT CC. The PCR product was gel purified and cloned into PCR Blunt (Invitrogen). The plasmid was amplified in One Shot Competent Cells (Invitrogen) and the N-RasV12 insert was excised with EcoRI and NotI, gel purified and sub-cloned into the pTracerSV40 vector, which was used for transfection of the cell lines following sequencing to check the orientation and fidelity of the N-RasV12 insert. Cells were seeded at 0.5×10^5 cells/ml in 35 mm dishes and cultured overnight in their regular media, prior to transfection. Transfection was performed using FuGene (Roche, Carlsbad, CA), according to the specifications of the manufacturer and transfected cells were selected using Zeocin (Invitrogen). Cells were examined for expression of GFP by immunofluorescent microscopy using a Zeiss Axiophot microscope and for the N-RasV12 gene by RT-PCR and Western blot as described below.

RT-PCR for N-RasV12

The presence of the N-RasV12 insert in transfected cells was confirmed by RT-PCR. Total RNA was extracted from a confluent cell monolayer using TriZol (Gibco BRL) according to the manufacturer's instructions. One microgram of total RNA was used for RT-PCR using the Titan One Tube RT-PCR kit (Roche). The primer pairs used were 5′ GAA TCC AGG ATG GCC ATG ACT GAG TAC AAA CTG GTG GTG and 3′ GCG GCC GCT TAC ATC ACC ACA CAT GGC AAT CC.

Conditions for all RT-PCR reactions were: denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 2 min for 30 cycles followed by a final extension at 72°C for 10 min. Integrity of the PCR DNA was checked by enzyme restriction analysis.

Western Blot

Expression of androgen receptors (ARs) and N-RasV12 protein was confirmed by Western blot. Confluent cell layers were washed once with 1 ml phosphate buffered saline (PBS) pH 7.4 and cells lysed in lysis buffer (0.15 M NaCl, 50 mM Tris-HCl pH 7.4, 1% Triton X-100, 1% deoxycholate, 0.4 mM Pefablock (Roche), and 3 μg/ml Pepstatin A (Sigma Chemical Company). Samples were electrophoresed through 7% (AR) or 14% (N-ras) sodium dodecyl sulphate (SDS)–polyacrylamide gels and then electroblotted onto a polyvinilidene difluoride membrane (Immobilon-P, Millipore Corp., Bedford, MA) with transfer buffer containing 10% (v/v) methanol. Blots were incubated overnight at 4°C with a 1:200 dilution of rabbit polyclonal anti-AR antibody (N-20, Santa Cruz, Inc., Santa Cruz, CA) or a 1:100 dilution of mouse monoclonal pan-ras Ab1 (Oncogene, San Diego, CA). Horseradish peroxidase (HRP)-labeled secondary antibody (sheep anti-rabbit or anti-mouse mouse IgG antibody, Amersham Life Science, diluted 1:10,000) was added for 1 hr at room temperature. Bands were visualized by enhanced chemiluminescence (Amersham Life Science).

Immunohistochemistry

Immunocyto- and immunohistochemistry were performed on cultured cells and tissue sections of tumors, respectively. Cells were seeded at 1×10^4 cells/well in 8-well Lab-Tek chamber slides (Nalge Nunc International, Naperville, IL) and cultured until 80% confluent, after which they were fixed and stained for cytokeratins 8/18 or 5/14 as described previously [42,43] or for a wide range of cytokeratins using a wide spectrum anti-cytokeratin antibody [44,45].

Tumor tissues were fixed in 70% ethanol or 4% paraformaldehyde and embedded in paraffin. Four micrometer sections were cut using a Spencer 820 microtome. Paraffin sections were deparaffinized in xylene and alcohol and rehydrated in PBS. Endogenous peroxidase was quenched by incubating with 0.5% H_2O_2 in PBS (v/v) for 30 min at 37°C. Sections were then incubated for 1 hr at room temperature in 50% goat serum in PBS containing 0.1% BSA (v/v) followed by incubation for 1 hr at room temperature with appropriate primary antibodies against cytokeratins or GFP. After washing, tissue sections were incubated with FITC-linked or HRP-linked secondary antibodies, followed by a second washing step and incubation with peroxidase substrate in the case of HRP-linked antibodies (NovaRed substrate kit for peroxidase, Vector, Burlingham, CA). Antibodies to cytokeratin 8 that were directly coupled to HRP, using the Dako EnVision + kit were also used to examine sections of tumors for cytokeratin 8 expression. GFP was detected using the Vectastain Elite ABC kit (Vector, Burlingham, CA), according to the manufacturer's instructions. Slides were counter-stained in

hematoxylin using standard methods and mounted with Permount. Positive controls included normal prostate sections for cytokeratin antibodies and prostate sections from a transgenic mouse expressing GFP in all of its tissues C57BL/6-TgN(ACTbEGFP) 10sb (The Jackson Laboratory, Bar Harbor, ME) for GFP antibodies.

Cell Proliferation Assays

We determined the effect of TGF-β (1, 0.1, and 0.01 ng/ml) and DHT (10^{-8}–10^{-12} M) on cell growth. Cells were seeded in duplicate wells in 24-well plates at 2×10^4 cells/well in DMEM with 10% FBS and allowed to adhere for 24 hr. For the proliferation assays performed to determine the effects of DHT, cells were seeded in DMEM containing 10% androgen-free charcoal-stripped fetal bovine serum [46]. After cell adherence, the medium was replaced with DMEM with 2% charcoal-stripped FBS and the appropriate concentrations of TGF-β or DHT. Cells were enumerated by counting every 24 hr on an Improved Neubauer hemocytometer.

Colony Formation Assays

Cells were cultured in soft agar to determine their ability to proliferate in an anchorage-independent manner. Five hundred microliters of appropriate medium and 500 μl of 1% agar at 50°C were mixed in sterile 15 ml conical tubes and added to 35 mm tissue culture dishes. Cells were seeded on this layer of solidified agar at 1×10^4/ml in 0.33% agar containing the appropriate media for each cell type. After the agar solidified, it was overlaid with 1 ml of the appropriate medium preferred by that cell type and placed at 37°C in a humidified incubator containing 5% CO_2. This medium was replaced with fresh medium after 1 week. Colonies of more than eight cells were counted after 14 days.

Measurement of TGF-β Production

Production of TGF-β by the normal cell lines and their transformed counterparts was measured using the transfected mink lung cell (TMLC) TGF-β bioassay [42,43,47]. Cells were seeded at 2×10^4 cells/ml in 35-mm dishes in their preferred media, which was replaced after 24 hr with 2 ml of DMEM containing 0.1% low endotoxin BSA. In the case of non-adherent cells, cells were seeded initially in DMEM containing 2% FBS to facilitate attachment of the cells prior to addition of the DMEM-BSA medium. Conditioned BSA medium was removed after 24 hr and added to the TMLC, either directly (for measurement of active TGF-β) or after heating at 80°C for 10 min (to convert latent TGF-β to the measurable active form). After 16–20 hr the TMLC were lysed and TGF-β was measured as described previously [42,43,47]. A TGF-β neutralizing monoclonal antibody (antibody 1D11.16; 20 μg/ml) was added to each sample of conditioned medium to confirm that the activity measured resulted from TGF-β activation. All experiments were performed in triplicate.

Tumor Formation in Athymic Mice

Tumorigenicity of the cell lines was determined after subcutaneous inoculation of cells from subconfluent monolayers of normal cell lines. Each cell line (5×10^6 cells/100 μl) was inoculated into the subscapular region of five 6 week-old male athymic mice. Athymic mice were used as GFP can elicit an immune response in syngeneic hosts [48]. The tumorigenic "basal" and "luminal" epithelial cells were inoculated alone or in conjunction with 2.5×10^6 normal prostatic SMC. Cells were inoculated into intact or castrated mice to determine the effect of androgens on tumor growth. Mice were examined twice weekly for at least 6 weeks for evidence of tumor growth and tumor volume was measured. Tumors were removed, fixed in 70% ethanol or 4% paraformaldehyde, and examined histologically and immunohistochemically for expression of cytokeratins and GFP. These experiments were repeated three times.

A transformed "basal" cell line, TE-B-1, was also injected at 0.5×10^6 cells/lobe/10 μl in the dorsolateral prostate of seven 6-week old male athymic mice. The cells were injected into both lobes of the dorsolateral prostate, using a dissecting microscope and a 30-guage needle. Mice were sacrificed after 6 weeks and the prostatic tumors were removed and their volumes recorded. Results were expressed as the mean +/− standard deviation (SD) and data were analyzed using the Student *t*-test. Tumors were examined histologically and immunohistochemically for the presence of GFP and "basal" and "luminal" cytokeratins.

RESULTS

Phenotype of Transformed Cell Lines

Three tumorigenic epithelial cell lines were derived by N-RasV12-transformation of the parental "basal" and "luminal" cell lines. One of these was "basal" in origin (line TE-B-1) and two were "luminal" in origin (lines TE-L-1 and TE-L-2). A third, spontaneously transformed "luminal" line (TE-L-4) was derived by passaging normal PE-L-1 cells through athymic mice. Although the PE-L-1 cell line was generally non-tumorigenic when inoculated subcutaneously in male athymic mice, in one animal a small subcutaneous tumor developed after 8 weeks. The cell line, TE-L-4, was derived from this tumor. The N-Ras-transforma-

tion was achieved by transfecting the parental cell lines with vector pTracerSV40-N-RasV12 that has both a GFP gene and the N-RasV12 proto-oncogene. The presence and activity of the N-RasV12 gene was confirmed in TE-B-1, TE-L-1, and TE-L-2 by RT-PCR and Western blot (data not shown). Lines TE-L-1, -2, and -4 were cultured in serum-free medium and line TE-B-1 was cultured in serum-containing medium, described previously [43].

The parental PE-B-1 "basal" line and transformed line TE-B-1 had a similar morphology (Fig. 1A,D). Cells were large, with many rounded cells floating free in the medium or adhering loosely to the monolayer. Like the parental cells, TE-B-1 cells expressed cytokeratin 5 (Fig. 1B,E) and 14 but not cytokeratins 8 or 18 (data not shown). Fluorescence could not be detected in fixed cells probed with normal control mouse or rabbit IgG (Fig. 1C,F), confirming that the fluorescence was due to specific FITC-label and not to the GFP expressed by the cells. GFP cannot be detected in cells fixed with methanol:acetone (50:50 v:v).

The adherent transformed TE-L-2 cells had a similar morphology to the parental "luminal" cell line, line PE-L-1, but in addition to the adherent cuboidal cells, there were also many round unattached cells growing in the medium (Fig. 1G,M). Like the parental line, TE-L-2 cells expressed cytokeratins 8 and 18 (Fig. 1H,N) but no basal cytokeratins (data not shown). The cells of lines TE-L-1 (Fig. 1J) and TE-L-4 (Fig. 1P) were round and refractile and grew in an anchorage independent manner in the absence of serum. In the presence of low serum (2% FBS) these cells attached to the culture dish, although they continued to grow in clusters rather than as a monolayer (not shown). Lines TE-L-1 (Fig. 1K) and TE-L-4 (Fig. 1Q) both expressed luminal cytokeratins and no basal cytokeratins.

Effect of DHTand TGF-β on Normal and Transformed Cell Lines

We examined the effect of androgens and TGF-β on the growth of the transformed lines as these factors were shown to affect growth of their normal counterparts [42,43]. Both the parental "basal" line, PE-B-1, and its transformed counterpart, TE-B-1, expressed ARs, as detected by Western blot analysis (Fig. 2A). Parental line PE-B-1 responded mitogenically to DHT, showing a 20% increase in growth rate in the presence of 10^{-12} M DHT [43]. The transformed "basal" line, TE-B-1, was more sensitive to the mitogenic effects of DHT than its parental line (PE-B-1), with growth being stimulated by 20–40% by 10^{-12}–10^{-8} M DHT (Fig. 2B). The PE-L-1 parental "luminal" line also expressed androgen receptors but did not respond to DHT. Growth of the transformed lines, TE-L-1, -2, and -4, was similarly unaffected by DHT (data not shown).

Growth of the parental "basal" line, PE-B-1, and the transformed "basal" line, TE-B-1, were unaffected by TGF-β (data not shown). TGF-β significantly inhibited the growth of the parental "luminal" epithelial cell line, PE-L-1. In the presence of 1 ng/ml of TGF-β, PE-L-1 growth is reduced by 42% after 6 days [43]. Tumorigenic lines TE-L-1, -2, and -4 showed varied responses to this cytokine. The growth of lines TE-L-1 and -2 was not significantly affected by TGF-β (Fig. 2C), indicating resistance to the inhibitory effect of this cytokine. However the growth of TE-L-4 was significantly inhibited by TGF-β, with growth being reduced by 70% in the presence of 1 ng/ml of the cytokine (Fig. 2C).

TGF-β Production and Activation

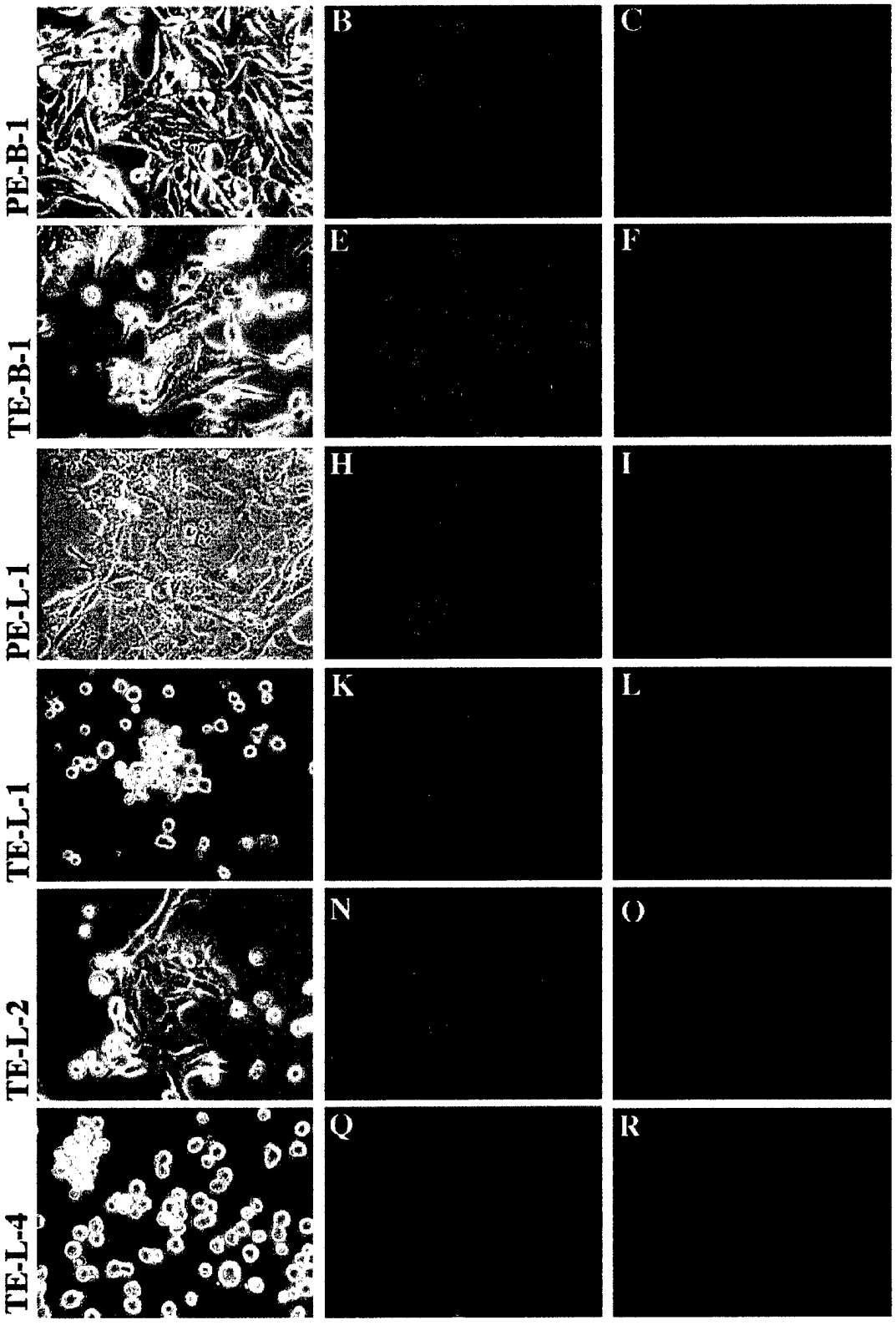
Tumor lines have been reported to lose sensitivity to TGF-β growth inhibition and to produce and activate increased amounts of TGF-β, which may enhance malignant progression [35]. The mink-lung luciferase assay for TGF-β quantifies both active TGF-β and latent TGF-β that has been converted into its biologically active form [42,43,47]. The parental "basal" cell line, PE-B-1, produced significant amounts of TGF-β [43]. The transformed "basal" line, TE-B-1, produced 220 pg/ml of latent TGF-β, significantly less than the 375 pg/ml produced by the parental line, PE-B-1 (Fig. 3). The parental "luminal" line, PE-L-1, produced low amounts of TGF-β [43]. Tumor lines TE-L-1 and -4 produced between 30 and 50 pg/ml of latent TGF-β, comparable to that produced by the parental line PE-L-1 (Fig. 3). Line TE-L-2 did not produce any measurable latent TGF-β. None of the cell lines produced any measurable active TGF-β (results not shown).

Growth in Soft Agar

The ability of cells to grow in an anchorage-independent manner is a characteristic of transformed cells. We therefore seeded the cell lines in 0.33% agar and counted colonies of more than eight cells after 2 weeks of culture. Both the parental, PE-B-1, and the transformed TE-B-1, "basal" cell lines had a low, comparable colony forming efficiency of 1% (Table I). The parental "luminal" cell line PE-L-1 did not form colonies in this assay [43]. However, all of the transformed "luminal" cell lines exhibited anchorage-independent growth, with a colony forming efficiency of 11–13% (Table I).

Subcutaneous and Orthotopic Tumor Formation

We examined the ability of the transformed epithelial cell lines to form tumors by inoculating the


PE-B-1
TE-B-1
PE-L-1
TE-L-1
TE-L-2
TE-L-4
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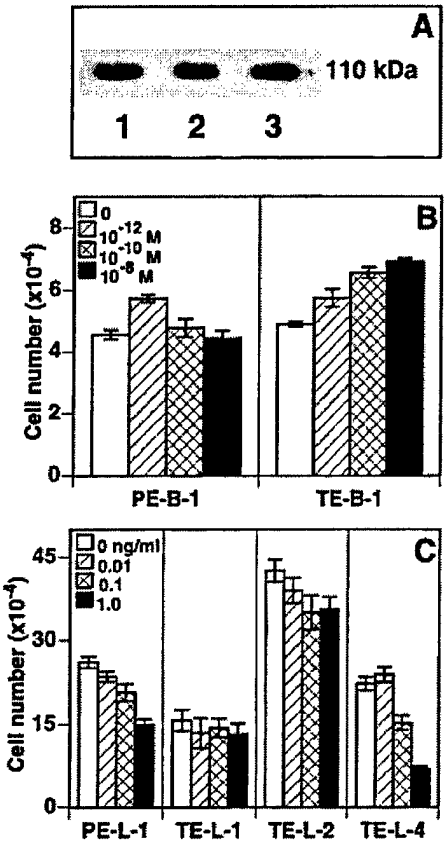

Fig. 2. **A**: Western blot analysis of androgen receptor (AR) protein. **Lanes 1** and **2**: AR protein detected in 100 μg of total cellular protein from cell lines PE-B-1 (parental "basal") and TE-B-1 (transformed "basal"), respectively. **Lane 3**: AR protein detected in 30 μg of total cellular protein from control LNCaP cells. **B**: Growth of the parental "basal" line, PE-B-1, and the transformed "basal" line, TE-B-1, in the absence and presence of DHT. Cells were seeded at 2×10^4 cells/well in 24-well plates in DMEM with 10% charcoal-stripped fetal bovine serum. After 24 hr, medium was replaced with DMEM containing 2% charcoal-stripped FBS and $10^{-8} - 10^{-12}$ M DHT and cells were counted after 4 days. Control wells received an ethanol vehicle. **C**: Growth of parental luminal cell line PE-L-1 and transformed lines TE-L-1, -2, and -4 in the absence and presence of TGF-β. Cells were seeded at 2×10^4 cells/well in 24 well plates in DMEM with 10% FBS. After 24 hr, medium was replaced with DMEM containing 2% FBS and 0.01 – 1 ng/ml TGF-β. Cells were counted after 6 days.

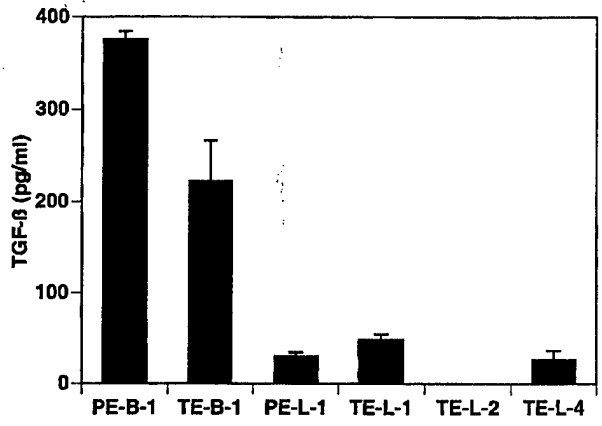

Fig. 3. Production of TGF-β by parental and transformed "basal" and "luminal" epithelial cell lines. Cells were seeded at 2×10^4 cells/ml in 35 mm dishes in their preferred media, which was replaced after 24 hr with 2 ml of DMEM containing 0.1% BSA. Conditioned medium was removed after a further 24 hr, heat activated at 80°C for 10 min and placed onto TGF-β-responsive mink lung epithelial cells (TMLC). After 20 hr, the TMLC were lysed and TGF-β was measured and quantified by comparison with a standard curve.

cells subcutaneously into athymic mice. All four transformed epithelial cell lines formed progressively growing subcutaneous tumors. The rate of tumor growth varied between the lines, with line TE-L-4 growing most rapidly, while tumors from TE-L-1 grew slowly (Fig. 4). All mice inoculated with transformed epithelial lines developed tumors within 3 weeks and animals were sacrificed within 6 weeks. Lungs and axiliary lymph nodes were removed from all mice and examined histologically for evidence of metastases but none of the lines proved to be metastatic. There was no significant difference in tumor growth rate or size in the presence (intact mice) or the absence (castrated mice) of androgens (not shown).

Previous reports have indicated that normal stromal cells such as fibroblasts can increase the tumorigenic potential of tumor cell lines in vivo [38,39,49]. In order to determine whether prostatic stroma could promote the growth of prostatic tumors we inoculated our tumor cell lines subcutaneously in the absence and presence of two normal prostatic smooth muscle

Fig. 1. Phenotype of parental epithelial cell lines, PE-B-1 and PE-L-1, and their transformed counterparts, TE-B-1, TE-L-1, -2, and -4. **A** and **D**: Phase contrast micrographs of PE-B-1 (A) and TE-B-1 (D) showing similar cobblestone appearance at confluence and large, round cells adhering loosely to the monolayer. **B** and **E**: Immunoflourescence micrographs showing expression of basal cytokeratin 14 by PE-B-1 (B) and TE-B-1 (E), using FITC-labeled anti-CK14 antibodies. **G**: Phase contrast micrograph of PE-L-1 showing polygonal morphology. **H**: Immunofluorescence micrograph showing expression of cytokeratin 8 by PE-L-1, using a FITC-labeled anti-CK8 antibody. **J** and **P**: Phase contrast micrographs of TE-L-1 (J) and TE-L-4 (P) showing round, refractile cells growing in an anchorage independent manner. **M**: Phase contrast micrograph of line TE-L-2 showing cells growing with typical epithelial polygonal morphology as well as non-adherent cells growing in an anchorage independent manner. **K**, **N**, and **Q**: Immunoflourescence micrographs showing transformed lines TE-L-1 (K), TE-L-2 (N), and TE-L-4 (Q) expressing cytokeratin 8. Each cell line was stained with an appropriate normal rabbit or mouse IgG (C, F, I, L, O, R). Magnification, × 400.

TABLE I. Colony Forming Efficiencies of Normal and Transformed Epithelial Cell Lines in Soft Agar

Cell line	Seeded cell number	Colonies (SD)	Efficiency (%)
PE-B-1	1×10^4	98 (11)	1.0
TE-B-1	1×10^4	94 (13)	1.0
PE-L-1	1×10^4	0	0
TE-L-1	1×10^4	1270 (37)	12.7
TE-L-2	1×10^4	1303 (31)	13.0
TE-L-4	1×10^4	1138 (109)	11.4

cell lines, PSMC1 [42] and PSMC2 (Fig. 5A,B). Prostatic smooth muscle was used rather than prostatic fibroblasts as prostatic epithelium is enveloped by SMC. Growth of tumors arising from TE-L-1 and TE-L-2 cells was not affected by PSCM1 or PSMC2 (data not shown). Tumors arising from TE-L-4 cells however were significantly stimulated by the presence of PSMC1 after 3.5–4 weeks ($P < 0.05$; Fig. 5A). Growth of tumors from line TE-B-1 was increased by both PSMC1 and PSCM2 ($P < 0.05$ and < 0.01; Fig. 5B). These results indicate that the growth of tumors that arose from the transformed "basal" cells (TE-B-1) and one of the transformed "luminal" cell lines (TE-L-4) was significantly enhanced by the presence of prostatic SMC.

The tumors arising from each transformed line inoculated alone were examined histologically. Tumors obtained from TE-B-1 had a uniform epithelial morphology (Fig. 6A). They expressed both GFP and

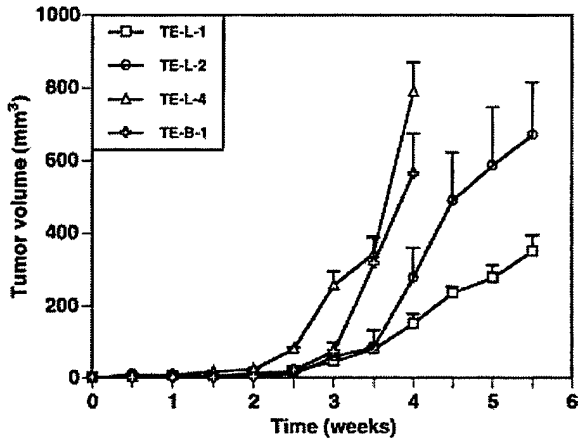

Fig. 4. Growth of subcutaneous tumors in athymic mice. Transformed cell lines, TE-B-1, TE-L-1, -2, and -4 were inoculated at 5×10^6 cells/100 µl in the subscapular region of 6-week-old male athymic mice. Tumor size was measured twice a week.

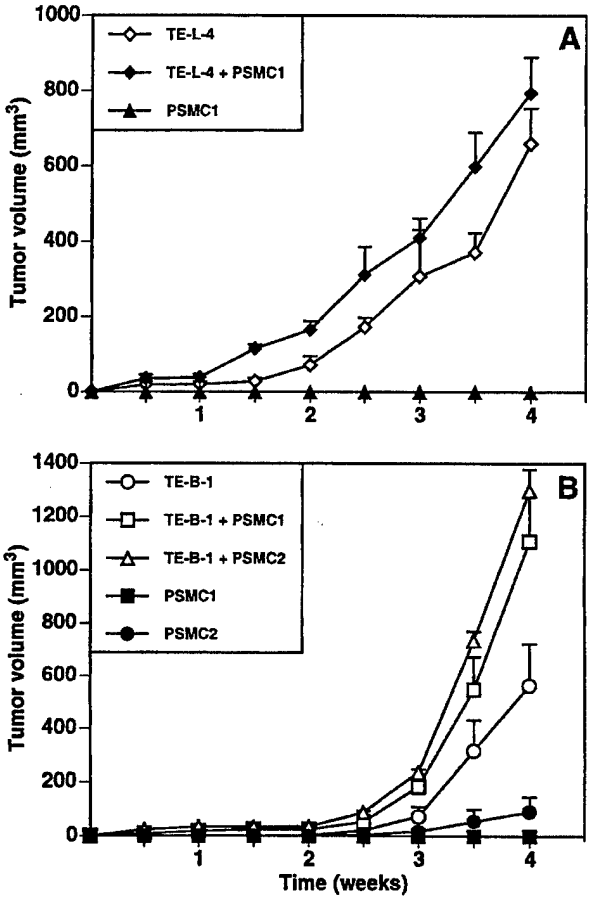

Fig. 5. Growth of subcutaneous tumors in the absence and presence of normal murine prostatic smooth muscle cells (SMC). Transformed cell lines TE-L-4 (**A**), TE-B-1 (**B**), were inoculated subcutaneously either alone (5×10^6 cells/100 µl), or in combination with prostatic SMC (lines PSCMI and PSCM2) (5×10^6 epithelial cells with 2.5×10^6 normal SMC/100 µl). Mice were examined twice weekly for evidence of tumor growth and tumor volume was measured.

basal cytokeratins at a low level, indicating their "basal" origin. They did not express luminal cytokeratins. Tumors arising from TE-L-1 and -2 were poorly differentiated with irregular epithelial cords interspersed with elongated stromal-like cells (Fig. 6B,C). The phenotype of tumors formed by line TE-L-4 was distinct in that they contained duct-like structures that were lined with cuboidal epithelial cells that strongly expressed luminal cytokeratins 8 and 18 (Fig. 6D,E). Tumors from lines TE-L-1 and -2 did not express either basal or luminal cytokeratins. All tumors obtained from transformed "luminal" cells contained GFP-expressing cells, although GFP expression was at a significantly lower level than found when the transformed cell lines were cultured in vitro.

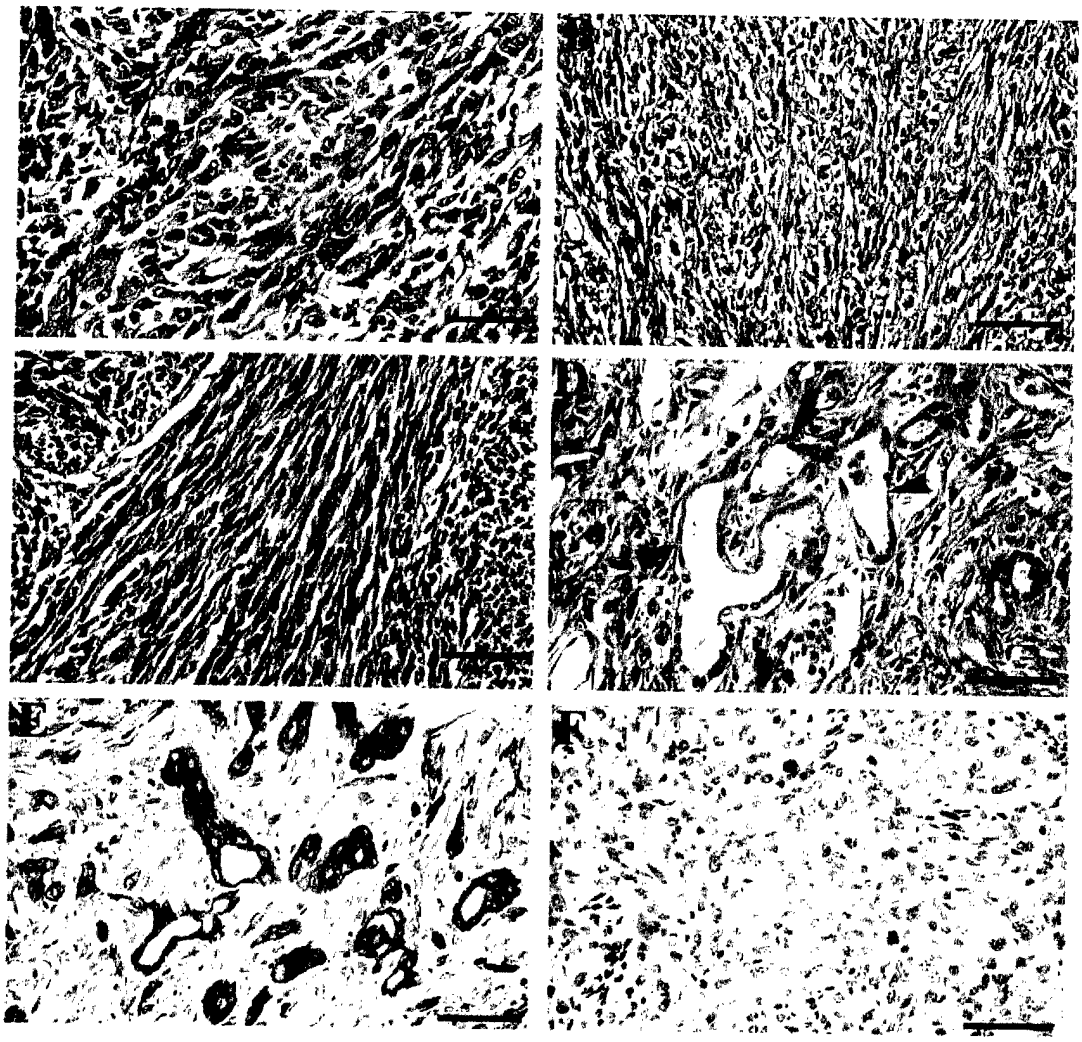

Fig. 6. Histology of subcutaneous tumors arising from transformed epithelial cell lines. **A**: Paraffin-section of a tumor arising from the transformed "basal" line, TE-B-1, showing uniform epithelial morphology. **B** and **C**: Paraffin-sections of tumors arising from transformed "luminal" lines TE-L-1 (B) and TE-L-2 (C) showing poorly differentiated tissue interspersed with irregular epithelial cords and elongated stromal-like cells. **D** and **E**: Paraffin sections of a tumor arising from TE-L-4 cells, showing ducts lined with epithelial cells (D-arrows), that strongly expressed luminal cytokeratin, CK 8 (E). Use of normal rabbit or mouse IgG indicated that the cytokeratin staining was specific (F). Bars = 100 μm.

The TE-B-1 "basal" cell line was inoculated orthotopically to determine the effect of intraprostatic inoculation on its growth. Tumors (Fig. 7A) were removed, fixed, and examined for cytokeratin expression and were found to express both "basal" (Fig. 7B) and "luminal" cytokeratins (Fig. 7C). This result indicates that transformed "basal" cells give rise to "luminal" cells when inoculated into a prostatic environment, confirming that "luminal" tumors may arise from transformed "basal" cells. Examination of the tumors using a wide-spectrum anti-cytokeratin antibody showed that the majority of the cells were epithelial in origin (Fig. 7E). Furthermore, the TE-B-1 origin of the cells in all tumors was confirmed by detecting GFP expression by immunohistochemistry. It could be shown that a majority of the cells within both subcutaneous tumors arising from inoculation of TE-B-1 cells in conjunction with prostate smooth muscle cells (Fig. 8A) and orthotopic tumors arising from TE-B-1 cells alone (Fig. 8C) expressed GFP. Since only the transformed epithelial cells and not the co-inoculated normal smooth muscle cells were transfected to express GFP, this result indicates that neither co-inoculated prostate smooth muscle cells nor host stromal cells contributed significantly to the tumor mass.

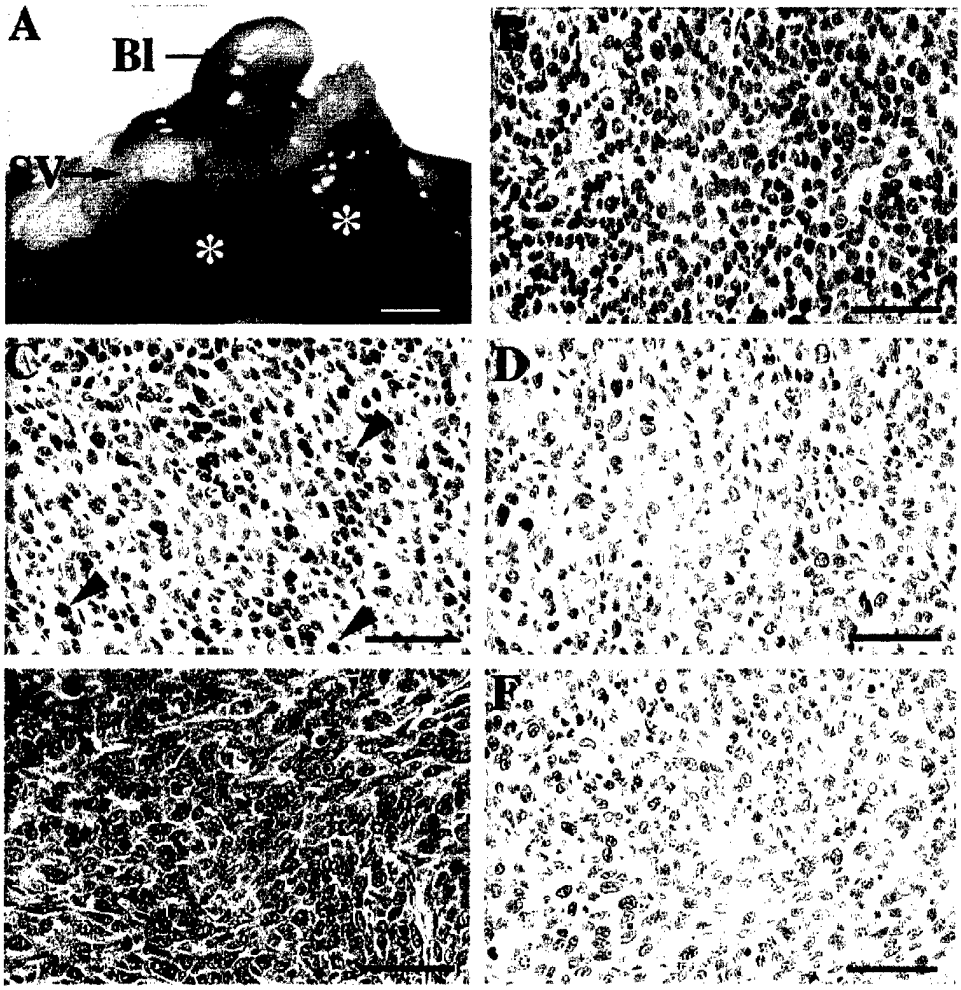

Fig. 7. Morphology and cytokeratin expression of intraprostatic tumors arising from the transformed "basal" cell line, TE-B-1. **A**: Gross morphology of intraprostatic tumors (*) growing in both lobes of the dorsolateral prostate. SV, seminal vesicle; Bl, bladder. Bar = 0.5 mm. Intraprostatic tumors arising from the transformed "basal" cell line were removed from sacrificed mice, fixed in 70% ethanol, embedded in paraffin and sectioned. Tissue sections were examined immunohistologically using antibodies to basal (CK14) and luminal (CK 8) cytokeratins. Tumors arising from lineTE-B-1 were found to contain cells expressing basal cytokeratins (**B**) and scattered luminal cytokeratins (C-arrows). Use of a wide spectrum anti-cytokeratin antibody confirmed that all the cells were epithelial in origin (**E**). Use of normal mouse or rabbit IgG indicated that the cytokeratin staining was specific (**D** and **F**). Bars = 100 μm.

DISCUSSION

Cell lines can provide powerful models for studying the acquisition and expression of the malignant phenotype. In this article we characterize the N-RasV12-transformed counterparts of a panel of normal murine prostatic "basal" and "luminal" epithelial cell lines in terms of their behavior in vitro and in vivo. Included in this panel is one spontaneously transformed luminal epithelial cell line. To our knowledge this is the first report of such a panel of cell lines derived from an inbred strain of mice.

We used the N-Ras oncogene to transform our normal "basal" (PE-B-1) and "luminal" (PE-L-1) prostatic cell lines. The normal parental cell lines were derived from p53 null C57BL/6 mice [42,43] and the absence of this gene in conjunction with the *ras* gene was sufficient to transform the cell lines. The frequency of Ras mutations in prostatic cancer in the United States is low and the mutations are primarily associated with advanced metastatic disease [50]. However 24% of prostatic carcinomas examined in Japanese men have Ras mutations [51,52]. The expression of Ras has been useful in studying prostatic carcinomas in murine models. Established immortalized rodent cell lines are readily transformed by the introduction of a single activated *ras* oncogene [21,22]. In addition it has been shown that the introduction of

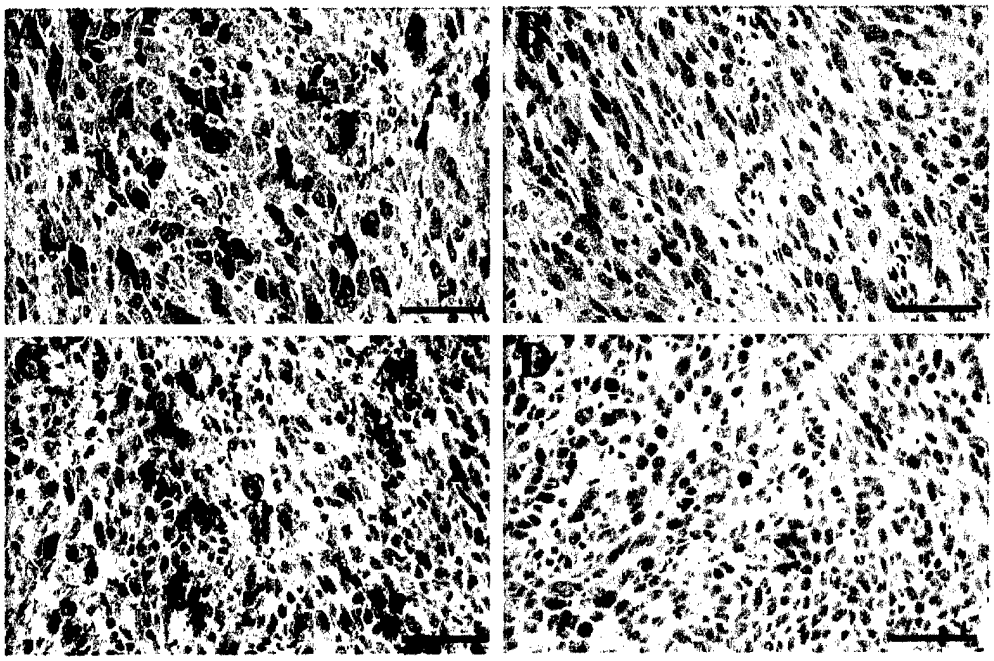

Fig. 8. Expression of GFP by cells in subcutaneous and orthotopic tumors. Subcutaneous and intraprostatic tumors were removed from sacrificed mice, fixed in 4% paraformaldehyde, embedded in paraffin and sectioned. Tissue sections were examined immunohistologically using antibodies to GFP. Most of the cells in both subcutaneous tumors arising from TE-B-1 cells inoculated with prostate smooth muscle cells (**A**) and orthotopic tumors arising from lineTE-B-1 inoculated alone (**C**) were found to express GFP, confirming that the tumors originated from the inoculated transformed TE-B-1 line. Use of normal mouse IgG indicated that the GFP staining was specific (**B** and **D**). Bars = 100 μm.

Ras into animals with a defective *p53* gene results in a dramatic increase in the malignant features of the transformed cells and in metastasis [23]. Since our parental lines were derived from p53 null mice and Ras has been used successfully to study prostatic carcinogenesis [23,54,55], we used transformation by Ras to derive our panel of transformed prostatic epithelial cell lines.

Normal, non-tumorigenic parental "basal" epithelial cells (line PE-B-1) were large round cells that expressed cytokeratins 5 and 14. At confluence, the cells formed islands with a typical epithelial cobblestone morphology (Fig. 1). The tumorigenic cell line (TE-B-1) also expressed cytokeratins 5 and 14 and exhibited a similar morphology to its parental line although the cultures contained significantly more round unattached cells (Fig. 1). DHT was mitogenic for both cell lines (Fig. 2B). TE-B-1 cells formed tumors when inoculated subcutaneously or orthotopically (Figs. 4 and 7). Although line TE-B-1 responds mitogenically to DHT in vitro, the cells are not androgen responsive in vivo, as tumor growth was not significantly altered in castrated animals. While TE-B-1 cells expressed significant amounts of basal cytokeratins in vitro, expression was diminished in the subcutaneous tumors, suggesting that the cells may have dedifferentiated in vivo. However, the cells of orthotopic tumors expressed high levels of basal cytokeratins, suggesting that cytokeratin expression may be enhanced by the prostatic environment. In addition, the intraprostatic tumors formed from the transformed "basal" line also contained cells that expressed luminal cytokeratins indicating that prostatic tumors expressing luminal cytokeratins can arise from transformed "basal" cells (Fig. 7). Therefore, although most human prostate carcinomas have a luminal phenotype, they may arise originally from transformed "basal" cells.

Normal parental "luminal" epithelial cells, line PE-L-1, grew in a characteristic cobblestone pattern and expressed cytokeratins 8 and 18 (Fig. 1). The tumorigenic lines, TE-L-1, -2, and -4, differed morphologically from the parental cell line. TE-L-2 resembled its parent in that attached islands of cells were present, but in addition, cultures contained many non-adherent cells. TE-L-1 and -4 grew as loosely attached round cells in culture. While both TE-L-1 and 2 continued to express cytokeratins 8 and 18 in culture, TE-L-4, a spontaneous transformant, expressed these cytokeratins in a far weaker and heterogeneous manner (Fig. 1). However, tumors that arose from TE-L-4 cells contained ducts that strongly expressed luminal

cytokeratins 8 and 18 indicating that this transformed cell line retained the ability to form prostatic ducts in vivo (Fig. 6). DHT had no effect on the growth of any of the "luminal" cell lines in vitro or in vivo. All three transformed lines were capable of anchorage-independent growth in soft agar and they formed subcutaneous tumors in vivo (Fig. 4).

There is compelling evidence to support the concept that a functional stromal response contributes to cancer progression [36], with previous reports indicating that stromal cells stimulate tumor progression [36–40]. Paracrine signaling between other cell types and carcinoma cells has an important role in tumor formation, affecting efficiency of tumor formation, tumor growth rate and size, invasiveness of the tumor and development of metastases [41]. As SMC are adjacent to prostatic epithelial cells, we inoculated our tumor lines subcutaneously in the presence and absence of normal prostatic SMC. We found that the growth of tumors arising from TE-B-1 and TE-L-4 cells was enhanced by the stromal cells, suggesting that prostatic smooth muscle may promote tumor development (Fig. 5). The mechanism by which the stroma contributes to tumorigenesis is unknown. It is possible that the presence of the SMC supports and stabilizes the injected epithelial cells during the inoculation procedure, facilitating their growth. None of the tumor lines proved metastatic in vivo.

A common feature of cancer cells is their autocrine production of cytokines and their unresponsiveness to growth suppressors [35]. TGF-βs are multifunctional modulators of cell growth, differentiation, and function and recent evidence indicates that they are implicated in the tumor suppressor pathway [56,57]. In its biologically active form, TGF-β generally exerts a growth-inhibitory effect on prostatic epithelial cells that may lead to the death of the cells [36,58–60]. In the case of stromal cells, TGF-β, may have either a stimulatory [42,61] or an inhibitory [60,62,63] effect on the growth of the cells. In the normal prostate TGF-β is considered to regulate growth through its antiproliferative effects while in tumorigenesis, TGF-β has a biphasic role, having inhibitory growth effects at early stages but at later stages enhancing both the tumorigenic process and invasion [64]. Loss of sensitivity to growth inhibition by TGF-β is linked to malignant progression [35,65]. We therefore examined our cell lines for their growth responses to TGF-β and for the endogenous production and activation of this cytokine. The growth of the normal and transformed "basal" cells was unaffected by TGF-β. We have discussed possible reasons for the lack of response of PE-B-1 to TGF-β previously [43]. Since the TE-B-1 line is derived from this parental line, it is not surprising that the transformed line would also be resistant to the growth-inhibitory effects of TGF-β. TGF-β is inhibitory for the growth of the parental "luminal" cell line (PE-L-1) [42] and one of the transformed lines (TE-L-4). Lines TE-L-1 and -2 were not significantly inhibited by TGF-β indicating that these lines had lost their sensitivity to TGF-β. None of the "basal" or "luminal" tumor cell lines produced or activated significantly more TGF-β than their normal parental cell counterparts.

CONCLUSIONS

We describe the characteristics of a panel of normal murine prostatic "basal" and "luminal" cell lines and their transformed counterparts. These cell lines have a number of interesting features. The rate of subcutaneous tumor formation of the transformed "basal" cell line and one of the "luminal" cell lines is considerably enhanced by the simultaneous inoculation of prostatic SMC. This may be relevant as prostatic smooth muscle lies adjacent to the prostatic epithelium and may contribute to the tumorigenic process. Furthermore, tumors arising from intraprostatic inoculation of transformed "basal" cells also contained cells expressing luminal cytokeratins, indicating that some of the transformed "basal" cells had differentiated to a luminal phenotype. This suggests that carcinomas with a luminal phenotype may arise from transformed "basal" cells. Finally, one of the tumorigenic "luminal" cell lines gave rise to tumors that formed ductal structures that strongly expressed cytokeratins 8 and 18. These cell lines will therefore be useful for studying the interactions between prostatic tumor cells and smooth muscle cells that promote tumorigenesis. They can also be used for investigating the manner by which transformed "basal" cells give rise to cells with luminal features.

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